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# Jayoti Vidyapeeth Women's University, Jaipur

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# DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR SIMULTANEOUS ESTIMATION OF TENELIGLIPTIN HYDROBROMIDE HYDRATE AND ROSUVASTATIN CALCIUM IN BULK AND TABLET DOSAGE FORM


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## A Synopsis

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**(PHARMACEUTICAL SCIENCES)**

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# 1. INTRODUCTION

Analytical chemistry is a vital part of pharmaceutical chemistry. It involves separating, identifying and determining the relative amounts of the component in the sample. Selection of a suitable analytical method for estimation of individual drug in any dosage form is a challenge for an analytical chemist. The method so selected should provide analytical data as accurate as required, technically sound, defensible with low limit of uncertainty and above all amenable to routine laboratory use and capable of being performed by personnel with minimal technical experience[1-3].

Analytical method validation is the next important step in justification andacceptability of an analytical method, after method development. It enables scientists to communicate scientifically and effectively on technical matters. Set standards of evaluation procedures for checking compliance and taking remedial measures. However, validation of equipment and analytical methods is necessary, not only due to regulations and accreditation standards, but also as prerequisite in terms of any good analytical practice and should be on going in the form of re-validation with method changes[4].

## 1.1 Chromatography

### 1.1.1 History[5]

The term chromatography (chrom means "color" and graphy means "to write") and chromatogram was coined by Michael Tswett, a Russian botanist who first separated and isolated chloroplast pigments from leaves by column adsorption chromatography.

### 1.1.2 Definition[5]

Chromatography is a physical method of separation in which components to be separated are distributed between two phases, one of which is fixed phase called as stationary phase while other which flows is called as mobile phase.

### 1.1.3 Principle[5]

Chromatography is based on differential migration. It involves a sample (or sample extract) being dissolved in a mobile phase (which may be a gas, a liquid or a supercritical fluid). The mobile phase is then forced through an immobile, immiscible stationary phase (which may be a solid, liquid, or solid/liquid mixture, the liquid being held stationary by a solid). The phases are chosen such that components of the sample have differing affinities for each phase. A component which is quite soluble in the stationary phase will take

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longer to travel through it than a component which is not very soluble in the stationary phase but very soluble in the mobile phase. As a result of these differences in mobility, sample components will become separated from each other as they travel through the stationary phase. This is called chromatographic development.

**High performance liquid chromatography**[6]

High-performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. High performance is the result of many factors:

- Very small particles of narrow distribution range and uniform pore size and distribution
- High pressure column slurry packing techniques
- Accurate low volume sample injectors
- Sensitive low volume detectors
- Good pumping systems

Compared with the classical techniques, HPLC is characterized by:

- Small diameter (2-5 mm), reusable stainless steel columns.
- Column packings with very small (3, 5 and 10 μm) particles and the continual development of new substances to be used as stationary phases.
- Relatively high inlet pressures and controlled flow of the mobile phase10-14.
- Precise sample introduction without the need for large samples.
- Automated standardized instruments.
- Rapid analysis and high resolution.

**1.2.1 Chromatographic separation mechanism**[7]

HPLC separations involve both the mobile phase and the stationary phase (usually materials of varying hydrophobicity chemically bonded to a solid support). The amount of water in an HPLC mobile phase will determine how strongly a hydrophobic analyte is

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repelled into the stationary phase and how well retained it is. The chemical nature of the stationary phase will also govern how strongly the analyte is retained.

For this reason, HPLC is a technique that is driven by the 'selectivity' achieved using two interacting phases.

### 1.2.2 Modes of chromatography[7]

The 'mode' of chromatography is usually defined by a combination of a certain stationary phase type with a certain mobile phase type.

**Table 1.1: Modes of chromatography**

| Types of compounds | Mode | Stationary phase | Mobile phase |
|---|---|---|---|
| Neutrals, Weak acids Weak bases | Reversed phase | C18, C8, C4 Cyano, amino | Water/organic modifiers |
| Ionics, bases, acids | Ion pair | C18, C8 | Water/organic ion-pair reagent |
| Compounds not soluble in water | Normal phase | Silica, amino, cyano, diol | Organics |
| Ionic / inorganic ions | Ion exchange | Anion or cation exchange resin | Aqueous / buffer counter ion |
| High molecular weight compounds, Polymers | Size exclusion | Polystyrene silica | Gel filtration -aqueous Gel permeation - organic |

**Table 1.2: Comparison between Normal and Reversed Phase Chromatography**

| Properties | Normal phase chromatography | Reverse phase chromatography |
|---|---|---|
| Stationary phase polarity | High | Low |
| Mobile phase polarity | Low – medium | Medium – high |
| Typical mobile phase | Heptane/$CHCl_3$ | $CH_3OH/H_2O$ |
| Order of elution | Least polar first | Most polar first |
| To increase retention | Decrease mobile phase polarity | Increase mobile phase polarity |
| | Increase polarity of sample | Decrease polarity of sample |
| Dominant type of interaction | Dipole – dipole | Hydrophobic |

### 1.2.4 Instrumentation[8]

**The basic components of a HPLC system**

- Mobile Phase Reservoir
- Solvent Delivery System
- The gradient controller

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- Solvent conditioning column
- Injectors
- Pre/Guard Column
- Analytical Column
- Detector
- Computers, Integrator or Recorder

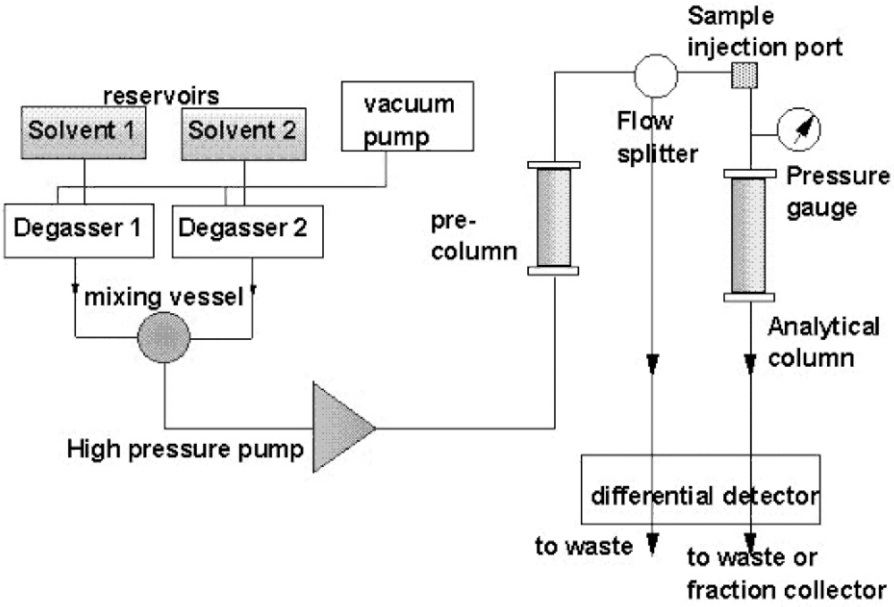


**Figure 1: Block diagram of a HPLC**

### 1.3.4 Parameters for analytical method validation [9,10]

1.3.4.1 Specificity

1.3.4.2 Linearity

1.3.4.3 Range

1.3.4.4 Accuracy

1.3.4.5 Precision

1.3.4.6 Detection Limit

1.3.4.7 Quantitation Limit

1.3.4.8 Robustness

1.3.4.9 System Suitability Testing

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## 2. REVIEW OF LITERATURE

**Hasumati A Raj *et al.* (2009),** has been developed and validated two new, simple, sensitive and accurate stability indicating methods for quantitative estimation of rosuvastatin in presence of degradation products in raw material. The first is HPLC method in which partition was accomplished on phenomenex C18 Column (250mm*4.6mm, 5.0 µm). The mobile phase selected for detection was acetonitrile: 0.5% formic acid (50:50, v/v) at a flow rate of 1.0 mL/min at ambient temperature (22-27C). The wavelength selected for detection at λmax 248 nm and concentration range of 5-300 µg/ml with mean recovery of 99.51-100.66%. The second method was HPTLC in which the drug is seperated from its acidic degradation products on silica gel 60F254 plates using mobile phase as a ethylacetate: toluene: acetonitrile: formic acid (6+ 3.5+ 0.5+ 0.2 v/v/v/v). The UV detection of the seperated band at 243 nm over a concentration range of 318-3816 ng/spot for rosuvastatin with recovery 99.77-101.94%. Both methods can analyse rosuvastatin in bulk with intraday and interday precision of 0.23-0.87% and 0.29-0.84%, respectively for HPLC method and 0.48-0.93% and 0.24-0.77%, respectively for HPTLC method. Developed methods can apply for the estimation of three strength of NOVASTAT tablet by Lupin pharmaceuticals[11]

**HO Kaila *et al.* (2010),**has been reported A new improved RP-HPLC method for assay of rosuvastatin calcium in tablets. The column used for isocratic separation of rosuvastatin was achieved on YMC C8 (5µm, 150 mm x 4.6 mm id column) at a flow rate of 1.5 ml/min. The PDA detector was used for detection at wavelength λmax 242 nm. The Acetonitrile and water (40:60, v/v) at pH 3.5 adjusted with phosphoric acid. The drug was subjected to oxidation, hydrolysis, photolysis and thermal degradation. The rosuvastatin eluting at approximately 5.2 min. The linearity of rosuvastatin was in concentration range of 0.5-80 µg/ml and correlation variable (r2) was 0.9993. The LOD and LOQ of rosuvastatin was found to be 0.1 and 0.5 µg/ml respectively. The recovery study of rosuvastatin was between 99.6 and 101.7%. The degradation product did not effect the detection of rosuvastatin and the assay is thus stability indicating. The developed method of rosuvastatin is sensitive and reliable[12].

**S Swathi *et al.* (2015),**has been validated RP-HPLC method for simultaneous determination of rosuvastatin calcium and ezetimibe in pharmaceutical dosage form. The

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column for separation of two drugs were achieved by using Enable C18G (5µm, 250 mm x 4.6 mm id column). The wavelength selected for UV detection at λmax 252 nm. The drugs were separated with a mobile phase acetonitrile and water (75: 25, v/v) at a flow rate of 0.6 ml/min. The linearity for both rosuvastatin calcium 5-40 µg/ml and ezetimibe were 5-40 µg/ml. A developed and validated was rapid, sensitive and specific for simultaneous determination of rosuvastatin calcium and ezetimibe in combined dosage form. The developed method can be used in quality control analysis of API and dosage form[13].

**Nita Yadav *et al.* (2017),** has been developed and validated teneligliptin in pharmaceutical dosage form by UV spectrophotometric methods. The solvent used for method development was distilled water. Three methods were used for estimation of teneligliptin in tablet dosage form.The λmax for quantitative estimation of teneligliptin for method I, method II and method III was selected at 244 nm, 266.4 nm and 238.6- 247.8 nm respectively. The method was developed and validated according to ICH guidelines. The linearity of teneligliptin for zero order and first order was in the range of 5-70 µorder and 5-80 µg/mL. The accuracy of teneligliptin tablet was ranged in between 98.54-101.80 for UV Spectrophotometric methods. The % assay of teneligliptin tablet by three methods was in the range of 100.17- 100.74. The regression coefficient (R2) was 0.999. The % RSD for all methods was ≤ 2. It was concluded that method has been developed and applied for quantitative estimation of pure drug of teneligliptin in pharmaceutical formulation[14].

**Dr. Ashti A mandale *et al.* (2017),** has been developed and validated a novel RP- HPLC method for the simultaneous determination of Rosuvastatin and Teneligliptin in bulk and in synthetic mixture. The chromatographic column Luna C18 100A0 (250mm* 4.6mm, 5 µm) kept at ambient temperature and the wavelength selected for detection of rosuvastatin at λmax 240nm. The compound eluted at a flow rate of 1.0 ml/min. The average retention time for teneligliptin and rosuvastatin were 2.583 and 5.458 min. The calibration curve were linear with correlation variable (r2) 0.9999over concentration range for teneligliptin and rosuvastatin were 10-200µg/ml and 10-200 µg/ml respectively. No chromatographic and spectral interferences from formulation excipients were found and successfully applied for determination of teneligliptin and rosuvastatin in bulk and in synthetic mixture. The recovery studies was found to be 98-101%. The methods was developed and validated according to ICH guidelines[15].

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**ME Hassouna *et al.* (2017),** has been developed a stability indicating new RP-HPLC method for the determination of rosuvastatin calcium in pure and tablets dosage forms. The wavelength selected for detection was at λmax 245nm. The chromatography column for separation of rosuvastatin calcium was achieved by using Agilent Eclipse XDB C8 (250mm x 4.6mm, 5 µm). The mobile phase used for separation by buffer solution of pH 4.5 containing 0.05M sodium dihydrogen phosphate: acetonitrile (50:50 v/v) at a flow rate of 1.2 ml/min. The total run time was found to be 5 min. Linearity of rosuvastatin calcium obtained in the range of 5-100 µg/ml and Rt values of rosuvastatin found to be 3.684 min with correlation coefficient (r2) 0.9995 and LOD, LOQ was found to be 1.50 µg/ml and 4.56 µg/ml respectively. The Accuracy and precision was 100±2% and less than 2% respectively. The forced degradation studies performed according to ICH guidelines. Hence the developed RP-HPLC method is new, specific, precise, simple, accurate and suitable for quality control of raw materials and finished products[16].

**Dr. Pradnya Lokhande *et al.*(2019),** has been developed and validated an analytical method of teneligliptin by using RP-HPLC with ICH guidelines. The wavelength for detection of teneligliptin at λmax 246nm with mobile phase methanol: phosphate buffer pH-3 (70:30, v/v). The linearity shows concentration range 10- 50µg/ml and correlation variable (r2) 0.9968. The method was developed and validated according to ICH guidelines. The LOD and LOQ was found to be 0.109µg/ml and 0.3305µg/ml respectively. The % purity of tablet formulation was found to be 99.56%. The % RSD of teneligliptin was found to be less than 2 %. The method which has been developed are simple, cheap, sensitive, less time consuming and appropriate for the determination of teneligliptin in bulk and pharmaceutical formulation by RP-HPLC[17].

**R Maruthi *et al.* (2020),**Worked on Analytical method development and validation of teneligliptin by RP-UFLC was fast, reliable, and cost effective method. The wavelength Used for detection using PDA detector was selected at 246 nm with flow rate 1.0 mL/min. The column selected for detection was phenomenex kinetex column C18 (250 mm X 4.6 mm 5 µm) with methanol and acetonitrile (60:40 v/v). The teneligliptin elutes at 4.938 min of processing period. The linearity of proposed method has 10-50 µg/ml and 0.9942% for the correlation variable (r2). Both LOD and LOQ was measured as 0.097 and 1.023 µg/mL, respectively. The pharmaceutical product has been under the stress condition of acidic, alkaline, degradation, ultraviolet and heat stress. The degradation

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studies were performed as per ICH guidelines with criteria of suitability, linearity, reliability, consistency and robustness[18].

**Rajani Vetapalem *et al.* (2020)**, has been developed and validated a stability indicating RP-HPLC method for simultaneous estimation of teneligliptin and metformin. The column used for analysis was kromasil C18 column (250*4.6mm, 5 µm) at 30 C with mobile phase, buffer: actonitrile: methanol (65:25:10 v/v/v). The wavelength selected for detection was at λmax 254 nm with a flow rate of 1.0 mL/ min. The linear graph for teneligliptin was in the range of 5-30 µg/mL and 125-750 µg/mL for metformin. The forced degradation studies were performed as per ICH guidelines of technical requirements for pharmaceuticals for human use under acidic, alkaline, oxidative, thermal, photostability and neutral conditions. Thus the developed method of teneligliptin and metformin in bulk and tablet dosage form was new simple, precise, specific and stability indicating using RP- HPLC[19].

**Bhanu Biswas *et al.* (2020),** Manish Kumar *et al.* was developed a new simple, specific, rapid, reliable and reproducible technique of RP-HPLC and validated for estimation of teneligliptin in tablet dosage form. The partition was accomplished on kromasil C18 analytical column (150mm*4.6mm, 5.0 µm) using acetonitrile (ACN), water and trifluoroacetic acid as mobile phase A and acetonitrile and trifluoroacetic acid as mobile phase B at a flow rate of 1.0 ml/min. The wavelength used for UV detector was detected at 245nm. The total chromatographic analysis time per sample was about 55.0 min with teneligliptin eluting at the retention time of about 11.2 minutes. The linearity was found to be linear in the concentration range of 50-150 µg/mL with R2 value of 0.999. The method was developed and validated for different parameters like accuracy, precision, specificity and linearity[20].

**Kallol Jana *et al.* (2020),**reported a reliable, sensitive, isocratic and simple RP-HPLC method was developed and validated for estimation of Rosuvastatin calcium in tablets and estimation of content uniformity. The thermo scientific C8 column, 250* 4.6mm, particle size 5 µm with flow rate of 1.0 ml/min. The wavelength selected for detection was at λmax 248nm. The mobile phase selected for detection was methanol: acetonitrile: water (40:40:20, v/v/v). The retention time of rosuvastatin calcium was found to be 3.427 and run time of approx 5 min. The linearity graph was linear within concentration range of

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140-260µg/ml with correlation variables (r2 = 0.999). The LOD and LOQ was 3.26 and 9.88 respectively. The percentage recovery of rosuvastatin calcium was between 106.58%, 100.18% and 102.81%. The developed method was validated for accuracy, precision, ruggedness, robustness and stability of solution. The method which was developed was accurate, simple, rapid, precise, time effective, reproducibleand can be applied routine quality control estimation of Rosuvastatin calcium in tablet dosage form[21].

**Saiyed Javedmiya Mukhtiyarmiya *et al.* (2021),** has been developed stability indicating analytical method development and validation for estimation of teneligliptin hemihydrate hydrobromide and rosuvastatin calcium in synthetic mixture. The good separation of analytes were achieved by using ethylacetate: toluene: acetonitrile: formic acid (6:3:0.5:0.2 v/v/v/v) with retardation factor (Rf) values of 0.37 ± 0.01 for rosuvastatin and 0.76 ± 0.01 for teneligliptin on precoated silica gel 60F254 plates. Rosuvastatin and teneligliptin, both were found to undergo alkali hydrolysis. The degradation of rosuvastatin and teneligliptin for with additional peaks at Rf values of 0.53 and 0.68 respectively. Both rosuvastatin and teneligliptin found to undergo acid hydrolysis. The acid hydrolysis showed degradation of rosuvastatin and teneligliptin for with additional peaks at Rf values of 0.48 and 0.62, respectively. Both were found to undergo oxidative reaction. In oxidative degradation study found that both drugs were very little to degradation without formation of any additional peak, both were subjected to dry heat condition. In dry heat degradation study, it was found that both drugs were stable and very little degradation occur without formation of additional peak. Photo stability study showed that teneligliptin were stable in sunlight. The proposed method was validated successfully and applied to determine rosuvastatin and teneligliptin hydrobromide hydrate in bulk and synthetic mixture. There is no eluting peak, hence the HPTLC method is specific for estimation of rosuvastatin and teneligliptin hydrobromide hydrate. The validated method were found to be accurate, precise, specific and cost effective[22].

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## 3. DRUG PROFILE

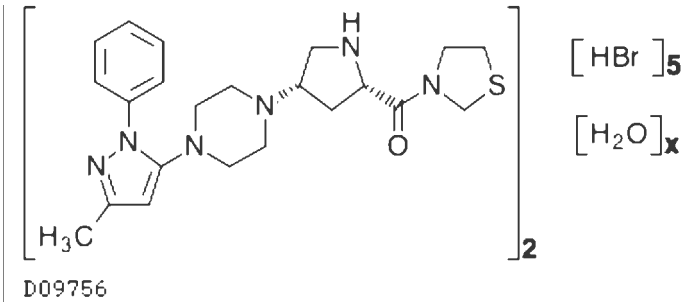
### 1. TENELIGLIPTIN HYDROBROMIDE HYDRATE:

Teneligliptin is a long-acting, that may reduce plasma triglyceride levels.

Molecular Formula: $(C_{22}H_{30}N_6OS)2.\ 5HBr.XH2O$

Molecular Weight: 1275.731 g/mol

Solubility: DMSO, Methanol, Water




**Mechanism of action:** Teneligliptin is a third generation DPP-4 inhibitor which in turn increases insulin secretion and decreases blood glucose levels.

### Medicinal Use:

Teneligliptin has been investigated for the treatment of Type 2 Diabetes Mellitus.

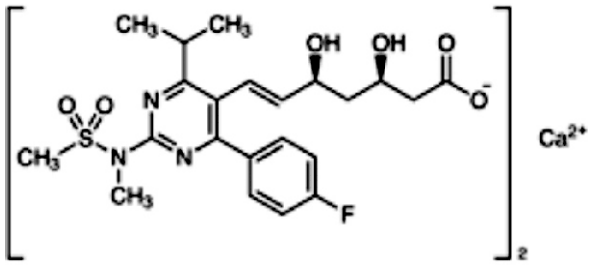
### 2. ROSUVASTATIN CALCIUM:

Rosuvastatin Calcium is the calcium salt form of rosuvastatin, a statin with anti-hyperlipidemic activity.

Molecular Formula: $C_{44}H_{54}CaF_2N_6O_{12}S_2$

Molecular Weight: 1001.1 g/mol

Solubility: Water




**Mechanism of action:** Rosuvastatin selectively and competitively binds and inhibits hepatic HMG-CoA reductase.

**Medicinal Use:**

- Lower the level of "bad" cholesterol (low-density lipoprotein, LDL).
- Lower the level of fat in blood (triglycerides).

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## 4. OBJECTIVES OF RESEARCH

- To develop and validate simple, rapid and valid methods.
- To determine assay of active ingredients in pharmaceutical formulation.
- To develop stability indicating analytical methods.
- To develop force degradation study under various stressed condition like acid, alkali.
- To develop analytical methods by using QbD approach.
- To develop a cost effective method.
- To develop and validate HPLC method for the simultaneous estimation of the Teneligliptin hydrobromide hydrate and Rosuvastatin calcium tablet.

## 5. RESEARCH HYPOTHESIS

Teneligliptin is an antidiabetic medicine used alone or in combination with other medicines to treat type 2 diabetes (a condition that results in high blood sugar) in adults. It works by increasing the activity of hormones that maintain blood sugar levels before or after a meal. It also increases insulin levels which helps to lower the sugar levels in your blood.

Rosuvastatin is used together with a proper diet to lower bad cholesterol (LDL) and triglycerides (fats) in the blood, and to increase your good cholesterol (HDL). It is also used to treat adults who cannot control their cholesterol levels by diet and exercise alone.

Combination of these drugs has been recently launched and it has been reported that teneligliptin co administered with Rosuvastatin is used in type 2 diabetes mellitus (T2DM) patients having dyslipidemia

No analytical and bioanalytical RP- HPLC methods have been developed for the simultaneous analysis of these drugs in combined dosage form. Hence, an attempt is made to develop analytical and bioanalytical method for simultaneous determination of teneligliptin and rosuvastatin in tablet dosage form.

The method which will be developed would have high recovery and low standard deviation that would show the suitability of method for routine quality control analysis in bulk and tablet dosage form in pharmaceutical industry.

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## 6. PLAN OF WORK

- Literature Review
- Selection of Drug
- Procurement of Drug and sample
- Solubility determination of drug
- Analytical method development by RP- HPLC
- Bioanalytical method development by RP-HPLC
- Analytical method development by using QbD approach
- Development of stability indicating analytical method
- Validation of developed method as per ICH Guidelines
    - Linearity
    - Specificity
    - Precision
    - Accuracy
    - Limit of detection
    - Limit of quantification
    - Robustness
- Result and Discussion
- Conclusion

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## 7. RESEARCH METHODOLOGY

1. **Physical Characterization of Drugs:** The drug would be physically characterized on the basis of appearance, color and odor at the beginning.
2. **Identification of Drugs:** The identification of drugs will be done by IR Spectroscopy.
3. **Melting Point determination of Drugs:** The melting point of drugs will be determined using open capillary method and compare the result with literature value.
4. **Selection of mobile phase:** Selection of suitable solvent depends on-
   a. Drugs must be soluble in solvent,
   b. Drugs must be stable in solvent,
   c. Drugs exhibit good spectral characteristics in it.
   d. They must be easily available, economical and of the HPLC grade
5. **Analytical method development by RP- HPLC:**

- **Determination of wavelength of both Drugs:** In order to select the wavelength to carry out the analysis, critical examination of the ultraviolet absorbance spectra of the drug should be done.
- **Preparation of calibration curve:** Prepare standard solution of different concentration from stock solution. Perform HPLC analysis for all standard solutions and record the peak area and retention time for each component. Plot calibration curve between Peak area vs. concentration using Ms. Excel. The correlation coefficient must be close to 1.
- **Determination of system suitability parameter:** System suitability parameter such as number of theoretical plate, Resolution, Retention time and tailing factor will be determined.

8. **Bio-analytical method development by RP-HPLC**

- Preparation of standard solution

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- Preparation of calibration curve
- Determination of drugs in plasma (Spiking method)

**10. Stability indicating RP- HPLC method:**

- Preparation of standard solution
- Assay procedure for tablet formulation
- Finally optimized chromatographic condition for method development
- Forced degradation study

**11. Method validation:** Validation of all the developed analytical method would be done by ICH guidelines. The parameter will be measured:

a. Linearity

b. Specificity

c. Accuracy

d. Precision

- Repeatability
- Intermediate Precision
- Day to Day Precision
- Analyst to Analyst Precision

e. Robustness

f. Limit of detection

g. Limit of Quantification

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